SpermMar IgA

SpermMar IgA Test

A qualitative beads test for detection of Sperm Antibodies of the IgA class

Preservative: Sodium azide 0.09%.
Store at 2° to 8° C - Do Not Freeze.
Reagent for Professional Use Only.

INTENDED USE

The SpermMar IgA test is a diagnostic kit for detecting antisperm antibodies of the IgA class in human semen. The presence of antisperm antibodies can interfere with sperm function and zona binding and the acrosome reaction.

GENERAL INFORMATION

The presence of sperm antibodies reacting with antigen(s) on the spermatozoa is considered as typical and specific for immunological infertility (1,3,22,24). These antibodies are found in approximately 8% of infertile men (13).
Antisperm antibodies belong to different immunological classes, but only those of the IgG and the IgA class are clinically relevant (18). The former display cytotoxic effects and are adequately detected on spermatozoa or in serum with the SpermMar IgG test. Antisperm antibodies when bound to complement C3 of the IgA class, which mainly have agglutinating properties (14), rarely occur without antibodies of the IgG class (6), but their meaning for male infertility may be more important. Indeed, patients combining sperm antibodies of the IgA class with IgG antibodies, or presenting IgA antibodies alone have very little chance of impregnating their partner through natural ways (6,20). Hence, detection of antibodies of the IgA class is of the utmost importance both for diagnosis and prognosis (21).
The bulk of the IgA class antisperm antibodies are secreted by the accessory sex glands (23). They are present on the spermatozoa and sometimes in seminal plasma, but usually are absent in serum. Therefore, testing for antisperm antibodies of the IgA class on serum is not recommended (18). It may be considered to search for sperm antibodies of the IgA class in seminal plasma, in cases with low sperm concentration or motility, although the possible clinical meaning of these antibodies is questionable.

The direct SpermMar IgA Test is a test for the detection of sperm coating antibodies, performed on either fresh spermatozoa, or spermatozoa which are isolated from seminal plasma by one cycle of suspension, centrifugation and resuspension in medium. These spermatozoa are mixed with the beads which are coated with antihuman anti-IgA. The formation of mixed agglutinates of motile spermatozoa with beads indicates the presence of IgA antisperm antibodies on the spermatozoa (1,5,9,10,17).

PRODUCT ORDER CODES

SPMA_S	SpermMar IgA single – 50 tests
SPMA_C	SpermMar IgA complete – 50 tests

MATERIALS INCLUDED WITH THE TEST

» 1 vial containing 0.7ml SpermMar IgA beads
» Microscope slides 76x26 mm*
» Cover glasses 24x40 mm*
» Microcapillary pipettes calibrated at 10 microlitres*
» Rubber bulb*

* complete kit only

MATERIALS NOT INCLUDED WITH THE TEST

» Light microscope (with 400x to 600x magnification, bright field, dark field or phase contrast)
» Non-spermicidal condom (e.g. Male Factor Pak - FertiPro NV)

DIRECTIONS FOR USE

SPECIMEN COLLECTION & PREPARATION

Semen collection by masturbation is preferred. Where particular circumstances discourage collection by masturbation, specific plastic condoms are available from FertiPro for semen collection (e.g. Male Factor Pak). Ordinary condoms should not be used for semen collection because they may interfere with the motility and viability of the spermatozoa. Ideally, semen should be examined within 1 hour after ejaculation.

REAGENT PREPARATION

The SpermMar IgA Latex particles are ready to use, however, they should be thoroughly mixed before use to provide a homogeneous suspension.

DIRECT SPERMMAR IGA TEST FOR THE DETECTION OF ANTISPERM ANTIBODIES OF THE IGA CLASS ON HUMAN SPERMATOZOA

1. Allow reagents and specimens to adjust to room temperature.
2. On a microslide place :
   » 10 microlitres of fresh semen
   » 10 microlitres of SpermMar IgA Latex particles
   This can be done by means of the 10 microlitres capillary pipettes, if provided in the kit.
   Note: To use the microcapillary pipettes, insert the end of the pipette marked with a heavy black line into the rubber bulb (approximately 5 mm). Allow pipette to fill by capillary action to the first mark (10 microlitres). Do not draw liquid into the bulb. Place tip of finger over the top of the bulb. Holding the bulb between thumb and middle finger, gently squeeze the bulb to expel the liquid from the pipette.
3. Mix the sample and the latex reagent 5 times with the edge of a cover glass.
4. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x to 600x magnification. The use of phase contrast or dark field illumination may facilitate reading the slide.
5. Read the result after 3 minutes. Observe for Latex particles attached to the motile sperm. Count 100 sperm cells to determine the percentage of reactive sperm. Read again after 10 minutes.
   Note: Keep the preparation in a damp chamber (e.g. a petri dish containing a moistened piece of filter paper).
6. The diagnosis of immunological infertility is suspected when 10-39% of the motile spermatozoa are attached to latex particles; if 40% or more of the spermatozoa are attached, immunological infertility is highly probable.

INTERPRETATION OF RESULTS

When the test is performed properly, the absence of antisperm antibodies will be shown by freely moving spermatozoa not covered by Latex particles. The Latex particles may, but usually do not agglutinate among themselves. In the presence of antisperm antibodies the spermatozoa will react with the particles and one, later several particles will attach to all or a proportion of the motile spermatozoa. The percentage of motile spermatozoa showing this mixed agglutination is directly related with the severity of the immunological reaction.
In general, the proportion of motile spermatozoa reacting in the SpermMar IgA test is smaller than that reacting in the SpermMar IgG test, but the contrary may occasionally occur (12). In rare cases there is a positive reaction in the SpermMar IgA test in the absence of any reaction in the SpermMar IgG test, indicating the presence of secretory antibodies of the IgA class without antibodies of the IgG class. Occurrence of mixed agglutination reaction of 40% or more in semen and 10% or more in cervical mucus indicate a positive reaction to the SpermMar IgA test.

LIMITATIONS OF THE METHOD

The direct SpermMar IgA test can only be performed if motile spermatozoa are present in the semen sample. Samples with very low sperm concentration or motility may yield false negative results.

PERFORMANCE CHARACTERISTICS

Several hundreds of semen samples have been tested with the direct mixed antiglobuline reaction and the direct SpermMar test for IgG. The results were similar in 97% of the cases. In 3% of the cases the SpermMar test detected antibodies whereas the mixed antiglobulin reaction test using coated red blood cells was negative. In such cases the proportion of spermatozoa reacting in the SpermMar test usually was low (14). The results of the SpermMar IgA test were proven accurate after comparison with immunofluorescence and nephelometry.

REAGENT STORAGE

When stored properly, SpermMar IgA reagent is stable for 12 months from date of manufacturing. SpermMar IgA reagent must be stored at between 2° and 8°C when not in use. DO NOT FREEZE REAGENT.

WARNINGS AND PRECAUTIONS

Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens. SpermMar IgA contains 0.1% Bovine Serum Albumin of US origin.

BIBLIOGRAPHY

1. BOETTCHER B., HJORT T., RUMKE Ph., SHULMAN S. and VYAZOV O.E. (eds.): Auto and iso-antibodies to antigens of the human reproductive system. 1. Results of an international comparative study of antibodies to spermatozoa and other antigens detected in sera from infertile patients deposited in the WHO Reference bank for reproductive immunology. Acta Pathol Microbiol Scand, 1977, 258 (suppl.) : 1-69
2. COMHAIRE F.H. and KUNNEN M.: Factors affecting the probability of conception after treatment of subfertile men with varicocele by transcatheter embolisation with Bucrylate. Fertil Steril, 1985, 43: 781-786.
3. FRIBERG J.: Immunoglobulin concentration in serum and seminal fluid from men with and without sperm-agglutinating antibodies. Obstet. Gynecol., 1980, 136 : 671-675.
4. GOLOMB J., VARDINON H., HOMONNAI Z.T., BRAF Z. and YUST I.: Demonstration of antispermatozoal antibodies in varicocele-related infertility with an enzym-linked Immunosorbent assay (ELISA). Fertil Steril, 1986, 45 : 397-402.
5. JAGER S., KREMER J. and VAN SLOCHTEREN-DRAAISMA T.: A simple method of screening for antisperm antibodies in the human male. Detection of spermatozoal surface IgG with the direct mixed antiglobulin reaction carried out on untreated fresh human semen. Int. J. Fertil., 1978, 23 : 12-21.
6. JAGER S., KREMER J., KUIKEN J. and VAN SLOCHTEREN-DRAAISMA T.: Immunoglobulin class of antispermatozoal antibodies from infertile men and inhibition of in vitro sperm penetration into cervical mucus, IntJ. Androl., 1980, 3: 1-14.
7. RUMKE P.: The origin of immunoglobulins in semen. Clin. Exp. Immunol., 1976, 17 : 287-297.
8. SHULMAN J.F. and SHULMAN S.: Methylprednisolone treatment of immunologic infertility in the male. Fertil Steril, 1982, 38 : 591-599.
9. STEDRONSKA J. and HENDRY W.F.: The value of the mixed antiglobulin reaction (MAR-Test) as an addition to routine seminal analysis in the evaluation of the subfertile couple. Am. J. Reprod. Immunol., 1983, 3 : 89-91.
10. VERMEULEN L. and COMHAIRE F.H.: Le test <MAR> aux particules de Latex, et le test spermatoxique selon Suominen : simplification et nouveauté dans l'arsenal du diagnostique immunologique. Contraception-Fertilité-Sexualité, 1983, 11 (suppl.) : 381-384.
11. W.H.O. 1984: Workshop on the standardized investigation of the infertile couple, moderator P. Rowe, coordinator M. Darling. Fertil Steril (eds) R.F. HARRISON, J. BONNAR and W. THOMPSON. Publ. M.T.P.-Press Ltd. (Lancaster, Boston, The Hague, Dordrecht), 1984, 427-431.
12. W.H.O.: Laboratory manual for the examination and processing of human semen. W.H.O., 5th Edition, 2010.
13. WORLD HEALTH ORGANIZATION: Towards more objectivity in diagnosis and management of male infertility. Int J Androl, 1987, Suppl 7.14. KREMER J. and JAGER S.: The significance of antisperm antibodies for sperm-cervical mucus interaction. Hum. Reprod., 1992, 7 : 781-784.
14. KAY D.J. and BOETTCHER B.: Comparison of the SpermMar test with currently accepted procedures for detecting human sperm antibodies. Reprod. Fer. Dev., 1992, 4, 175-181.
15. ACKERMAN S., Mc GUIRE G., FULGHAM D.L. and ALEXANDER N.: An evaluation of a commercially available assay for the detection of antisperm antibodies. Fertil Steril 1988, 49 : 732-734.
16. BRONSON R, COOPER G and ROSENFELD D: Sperm antibodies: their role in infertility. Fertil Steril, 1984, 42: 171-183.
17. CLARKE GN, STOJANOFF A, CAUCHI MN and JOHNSTON WIH: The immunoglobulin class of antispermatozoal antibodies in serum. Am J Reprod Immunol Microbiol, 1985, 7: 143-147.
18. HINTING A, VERMEULEN L and COMHAIRE F: The indirect mixed antiglobulin reaction test using a commercially available kit for the detection of antisperm antibodies in serum. Fertil Steril, 1988, 49 :1039-1044.
19. MEINERTZ H and HJORT T: Detection of autoimmunity to sperm: mixed antiglobulin reaction (MAR) test or sperm agglutination? A study on 537 men from infertile couples. Fertil Steril, 1986, 46 : 86-91.
20. PARSLOW JM, POULTON TA, BESSER GM and HENDRY WF: The clinical relevance of classes of immunoglobulins on spermatozoa from infertile and vasectomized males. Fertil Steril, 1985, 43 : 621.
21. RUMKE P and HELLINGA: Autoantibodies against spermatozoa in sterile men. Am J Clin Pathol, 1959, 32 : 357-363.
22. UELING DT: Secretory IgA in seminal fluid. Fertil Steril, 1971, 22 : 769-773.
23. WILSON L: Sperm agglutinins in human semen and blood. Proc Soc Exp Biol Med, 1954, 85 : 652-655.

TECHNICAL SUPPORT

FertiPro N.V.
Industriepark Noord 32, 8730 Beernem, Belgium
Tel +32 (0)50 79 18 05
Fax +32 (0)50 79 17 99
URL: www.fertipro.com
E-mail: info@fertipro.com

CE IVD

Doc. reference: FP09 I01 R01 E.1
Edition: 25.11.2014

SpermMarTest_IgA_IgG_07.11.14.indd 1 25/11/14 11:22

SpermMar IgG

SpermMar IgG Test

A qualitative Latex Test for detection of Sperm Antibodies.

Preservative: Sodium azide 0.09%.
Store at 2° to 8° C - Do Not Freeze.
Reagent for Professional Use Only.

INTENDED USE

The SpermMar IgG test is a diagnostic kit for detecting antisperm antibodies of the IgG class in human semen, cervical mucus or serum. The direct SpermMar IgG test can be performed on untreated human semen provided it contains motile spermatozoa, the indirect SpermMar IgG test can be used on serum and cervical mucus.
The presence of antisperm antibodies can interfere with sperm function and zona binding and the acrosome reaction.

GENERAL INFORMATION

The presence of sperm antibodies reacting with antigens on the spermatozoa is considered as typical and specific for immunological infertility (2, 4, 11). These antibodies are found in approximately 8% of infertile men (13).
Sperm antibodies belong to two immunological classes; IgA and IgG antibodies. There are some data indicating IgA to be more clinically important than IgG antibodies. However, IgA antibodies rarely occur without IgG antibodies. Therefore, testing for IgG antibodies is sufficient for routine screening (6,7,14).
The direct SpermMar IgG Test is performed by mixing fresh, untreated semen with latex particles that have been coated with human IgG. To this mixture a monospecific antihuman IgG antiserum is added. The formation of agglutinates between particles and motile spermatozoa indicates the presence of IgG antibodies on the spermatozoa (1,5,9,10). In the Indirect SpermMar IgG Test washed motile donor spermatozoa are incubated with diluted and de-complemented patient serum of male or female origin.
If the serum contains antisperm antibodies, these will cover the donor spermatozoa which will react positively in a subsequent SpermMar IgG Test.

PRODUCT ORDER CODES

SPMG_S	SpermMar IgG single – 50 tests
SPMG_C	SpermMar IgG complete – 50 tests

MATERIALS INCLUDED WITH THE TEST

- 1 vial containing 0.7 ml SpermMar IgG Latex Particles
- 1 vial containing 0.7 ml SpermMar IgG Antiserum
- Micro Slides 76 x 26 mm*
- Cover-glasses 24 x 40 mm*
- Microcapillary pipettes calibrated at 10 microlitres*
- Rubber bulb*

* complete kit only

MATERIALS NOT INCLUDED WITH THE TEST

- Light microscope (with 400x to 600x magnification, bright field, dark field or phase contrast)
- EBSS medium without added protein for the indirect SpermMar IgG test (e.g. Sigma-Aldrich - E2888)
- Cervical mucus aspiration device (e.g.In/Out - Gynetics, Belgium; Spirette™ - CCD, France; Aspiglaire™ - CBS, France)
- Non spermicidal condom (e.g. Male Factor Pak – FertiPro NV)
- Microtiter plate (e.g. Kima 650 101)

DIRECTIONS FOR USE

SPECIMEN COLLECTION & PREPARATION

Semen collection by masturbation is preferred. Where particular circumstances discourage collection by masturbation, specific plastic condoms are available from FertiPro for semen collection (e.g. Male Factor Pak). Ordinary condoms should not be used for semen collection because they may interfere with the motility and viability of the spermatozoa. Ideally, semen should be examined within 1 hour after ejaculation. Cervical mucus can be aspirated using a device specially designed for this purpose (e.g. In/Out - Gynetics, Belgium; Spirette™ - Laboratoire CCD, France; Aspiglaire - CBS, France).

REAGENT PREPARATION

SpermMar IgG Latex Particles are ready to use, however, they should be thoroughly mixed before use to provide a homogeneous suspension. SpermMar IgG Antiserum is ready to use.

DIRECT SPERMMAR IGG TEST

1. Allow the reagents and specimens to adjust to room temperature.
2. On a micro slide place :
   - » 10 microlitres of fresh untreated semen
   - » 10 microlitres of SpermMar IgG Latex Particles
   - » 10 microlitres of SpermMar IgG Antiserum

   This can be done by means of the provided 10 microlitres capillary pipettes (complete test).
   Note: To use the microcapillary pipettes: Insert the end of the pipette marked with the heavy black line into the rubber bulb (approximately 5 mm). Allow the pipette to fill by capillary action to the first mark (10 microlitres). Do not draw liquid into the rubber bulb. Holding the bulb between the thumb and the middle finger, gently squeeze the bulb to expel the liquid from the pipette.
3. Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
4. Mix the Antiserum with the Latex reagent and sample mixture.
5. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x or a 600x magnification (phase contrast or dark field illumination may facilitate reading of the slides).
6. Read the result after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm.
   If no attachment of beads to sperm is observed, read again after 10 minutes.

Note: Keep the preparation in a damp chamber (e.g. a Petri dish containing a moistened piece of filter paper).

INDIRECT SPERMMAR IGG TEST

1. Allow all reagents and specimens to adjust to room temperature.
2. Inactivate the serum specimens by heating them at 56°C for 30 minutes if glass test-tubes are used, 45 minutes if plastic test-tubes are used.
3. Adjust the pH (by adding 0.1N NaOH or HCl) of the EBSS to 7.4 - 7.5.
4. Wash the motile donor spermatozoa by letting them swim up in the pH adjusted medium (pH = 7.4 - 7.5). Swim up has to be done in 5 ml glass or sterile plastic test-tubes with round bottom at 37°C for 45 minutes Adjust the sperm concentration to $20x10^6$ sp/ml with EBSS medium (pH = 7.4 - 7.5)
5. Serially dilute the inactivated serum specimen 1/16 with EBSS medium (pH = 7.4 - 7.5) in a titre plate
6. Mix 50 microlitres of the (1/16) diluted, inactivated serum specimen (step 5) with 50 microlitres of the washed motile donor sperm (step 4) in a free well on the titre plate. Incubate for 60 minutes at 37°C.
7. On a micro slide place :
   - » 10 microlitres of the sperm-serum mixture
   - » 10 microlitres of SpermMar IgG Latex Particles
   - » 10 microlitres of SpermMar IgG Antiserum
8. Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
9. Mix the Antiserum with the Latex reagent and sample mixture.
10. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x or 600x magnification (phase contrast or dark field illumination may also be used to facilitate reading)
11. Read the results after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm.
    If no attachment of particles to sperm is observed, read again after 10 minutes.

Note: Keep the preparation in a damp chamber (e.g. a Petri dish containing a moistened piece of filter paper). To prevent evaporation during incubation, always cover with Parafilm.

RESULTS

When the test is properly performed, the absence of sperm antibodies will be shown by freely moving spermatozoa not covered by latex particles. The latex particles themselves will form growing agglutinates thus proving the reactivity of the reagents. In the presence of sperm antibodies however, the spermatozoa will be partially covered by latex particles. In some cases the spermatozoa might even be immobilized by the massive amount of adherent latex particles. In the direct SpermMar IgG Test, the diagnosis of immunological infertility is suspected when 10-39% of the motile spermatozoa are covered by latex particles; if 40% or more of the spermatozoa are covered, immunological infertility is highly probable. Additional tests should confirm the diagnosis. Whenever a positive result is obtained it is recommended to perform the SpermMar IgA test. In the indirect SpermMar IgG Test, the occurrence of 40% or more reaction between the coated latex particles and motile spermatozoa is generally accepted as the lower limit of significant activity.

LIMITATIONS OF THE METHOD

The direct SpermMar IgG Test can only be performed if motile spermatozoa are present in the semen. Samples with poor motility may yield false negative results in those cases it is suggested to perform the indirect SpermMar IgG test.

PERFORMANCE CHARACTERISTICS

DIRECT SPERMMAR IGG TEST

Several hundreds of semen samples were tested with the direct MAR-Test (mixed antiglobulin reaction based on red blood cells) and with the SpermMar IgG Test. The results were similar in 97% of the cases. In 3% of the cases the MAR-Test based on red blood cells was negative while the SpermMar IgG Test detected antibody coated spermatozoa, though in relatively small numbers (<40%), thus proving the higher sensitivity of the SpermMar IgG Test (10,16).

INDIRECT SPERMMAR IGG TEST

Using the value of 40% reaction between motile spermatozoa and coated Latex particles as the lower limit of significant activity, the indirect SpermMar IgG Test was found positive in some cases with negative results of the Tray Agglutination Test, or other currently accepted procedures.
The SpermMar IgG Test was proven easier to use and more sensitive (15). A false negative indirect SpermMar IgG Test in comparison with the Tray Agglutination Test occurred in cases with IgM in serum, the clinical significance of which is doubtful. It is recommended to confirm a positive result of the indirect SpermMar IgG Test by additional tests for the detection of agglutinating activity (Tray Agglutination Test) and of cytotoxic activity, such as the ATP-Release cytotoxicity test. The latter tests will also assess the type of immunological effect exerted by the antisperm antibodies.

REAGENT STORAGE

SpermMar IgG test reagents are stable for 18 months from the date of manufacturing. SpermMar IgG test reagents must be stored at 2° to 8°C when not in use. DO NOT FREEZE.

WARNINGS AND PRECAUTIONS

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens.
SpermMar IgG latex particles contains 0.1% Bovine Serum Albumin of US origin.
SpermMar IgG latex particles are coated with human IgG, all materials used have been tested by their original manufacturer for Hepatitis B, Hepatitis C and HIV.

SpermMar IgG Test positive and negative controls

Controls for use with the Indirect Test for Determination of Sperm Antibodies (SpermMar IgG)

Preservative: Sodium azide 0.09%.
Store at 2° to 8° C - Do Not Freeze.
Reagent for Professional Use Only.

INTENDED USE

The SpermMar IgG positive and SpermMar IgG negative control are designed to be used as quality control with the SpermMar IgG Test.

GENERAL INFORMATION

The presence of sperm antibodies reacting with antigens on the spermatozoa is considered as typical and specific for immunological infertility (2,4,11). Sperm antibodies belong to two immunological classes; IgA and IgG antibodies. There are some data indicating IgA to be more clinically important than IgG antibodies. However, IgA antibodies rarely occur without IgG antibodies. Therefore, testing for IgG antibodies is sufficient as a routine screening method (6,7,14).
In the Indirect SpermMar IgG Test washed motile donor spermatozoa are incubated with diluted, de-complemented patient serum of male or female origin. If the serum contains antisperm antibodies, these will cover the donor spermatozoa which will react positively in a subsequent SpermMar IgG Test.
The SpermMar IgG positive control contains ready-to-use patient serum with antisperm antibodies levels higher than 80%. The SpermMar IgG negative control contains ready-to-use patient serum with antisperm antibodies levels of below 20%.

PRODUCT CODES AND KIT CONTENTS

SPMG_P	1 vial with 2.5 ml of positive control serum for the SpermMar IgG test
SPMG_N	1 vial with 2.5 ml of negative control serum for the SpermMar IgG test

MATERIAL INCLUDED WITH THE TEST

- 1 vial with 2.5ml de-complemented patient serum diluted in FertiCult Flushing medium without human serum albumin

MATERIALS NOT INCLUDED WITH THE TEST

- SpermMar IgG Test
- Microscope slides
- Cover glasses
- Light microscope (with 400x to 600x magnification, bright field, dark field or phase contrast)
- EBSS medium (e.g. Sigma-Aldrich – E2888)
- Microtiter plate (e.g. Kima 650 101)

DIRECTIONS FOR USE

REAGENT PREPARATION

SpermMar IgG Positive and Negative control are ready to use. Allow to adjust to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

The donor semen should be collected by masturbation or by other methods recommended by the physician. Preferentially, semen should be examined within 1 hour after ejaculation.

PROCEDURE

1. Allow all reagents and specimens to adjust to room temperature.
2. Wash the motile donor spermatozoa by letting them swim up in the pH adjusted EBSS medium (pH = 7.4 - 7.5). Swim up has to be done in 5 ml glass or sterile plastic test-tubes with round bottom at 37°C for 45 minutes. Adjust the sperm concentration to $20x10^6$ sp/ml with medium (pH = 7.4 - 7.5).
3. Mix 50 microlitres of control serum with 50 microlitres of the washed motile donor sperm in a free well on the microtiter plate. Let incubate for 60 minutes at 37°C.
4. On a micro slide place :
   - » 10 microlitres of the sperm-serum mixture
   - » 10 microlitres of SpermMar IgG Latex Particles
   - » 10 microlitres of SpermMar IgG Antiserum
5. Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
6. Also mix the Antiserum with the Latex reagent and sample mixture.
7. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x or 600x magnification (phase contrast or dark field illumination may also be used to facilitate reading).
8. Read the results after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm. If no attachment of particles to sperm is observed, read again after 10 minutes.

Note: Keep the preparation in a damp chamber (e.g. a Petri dish containing a moistened piece of filter paper). To prevent evaporation during incubation, always cover with Parafilm.

RESULTS

When the test is properly performed, the absence of sperm antibodies will be shown by freely moving spermatozoa not covered by latex particles. The latex particles themselves will form growing agglutinates thus proving the reactivity of the reagents. In the presence of sperm antibodies however, the spermatozoa will be partially covered by latex particles. In some cases the spermatozoa might even be immobilized by the massive amount of adherent latex particles.

- » The SpermMar IgG positive control test should yield 80% or more of the motile spermatozoa covered with latex particles.
- » The SpermMar IgG negative control should yield 20% or less spermatozoa covered with latex particles.

LIMITATIONS OF THE PROCEDURE

The indirect SpermMar IgG Test can only be performed if motile spermatozoa are present in the semen.

REAGENT STORAGE

When stored properly, SpermMar IgG control sera are stable for 18 months from the date of manufacturing. SpermMar IgG control sera must be stored at 2° to 8°C when not in use.

WARNINGS AND PRECAUTIONS

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens.
Although SpermMar IgG Positive and Negative controls have been tested for HIV and hepatitis the user should always wear protective clothing when handling the control sera.

BIBLIOGRAPHY

See reverse side.

TECHNICAL SUPPLIER

FertiPro N.V.
Industriepark Noord 32, 8730 Beernem, Belgium
Tel +32 (0)50 79 18 05
Fax +32 (0)50 79 17 99
URL: www.fertipro.com
E-mail: info@fertipro.com

CE IVD

Doc. reference: FP09 I01 R01 E.1
Edition: 25.11.2014

SpermMarTest_IgA_IgG_07.11.14.indd 2 25/11/14 11:22